**QUEEN'S
UNIVERSITY
BELFAST**

Wheat powdery mildew and foliar N concentrations as influenced by N fertilization and belowground interactions with intercropped faba bean

Chen, Y. X., Zhang, F. S., Tang, L., Zheng, Y., Li, Y. J., Christie, P., & Li, L. (2007). Wheat powdery mildew and foliar N concentrations as influenced by N fertilization and belowground interactions with intercropped faba bean. *Plant and Soil*, 291(1-2), 1-13. <https://doi.org/10.1007/s11104-006-9161-9>

Published in:
Plant and Soil

Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Wheat powdery mildew and foliar N concentrations as influenced by N fertilization and belowground interactions with intercropped faba bean

Yuanxue Chen · Fusuo Zhang ·
Li Tang · Yi Zheng · Yongjie Li ·
Peter Christie · Long Li

Received: 10 August 2006 / Accepted: 2 November 2006 / Published online: 17 January 2007
© Springer Science+Business Media B.V. 2007

Abstract Wheat (*Triticum aestivum* L.) production by intercropping with faba bean (*Vicia faba* L.) has increased in popularity but is often associated with severe wheat powdery mildew (*Blumeria graminis* (DC.) Speer). Very little is known about the effects of below- and aboveground interspecific interactions on wheat nitrogen (N) nutrition and occurrence of wheat powdery mildew. A greenhouse pot experiment examined four N application rates and three belowground partition types (plastic film, nylon mesh partition or no partition) to study N nutrition and interactions between wheat and faba bean growing together. A field experiment investigated three N application rates and growth of wheat in monoculture and intercropped with faba bean with or without belowground plastic

film partitions between wheat and faba bean. Disease incidence (DI) and disease severity index (DSI) were assessed at flowering stage and wheat leaves were sampled and analyzed for N. Foliar N was enhanced substantially by N addition in greenhouse and field conditions and also by belowground interactions (no partition compared with plastic film partition) in the pot experiment (all $P < 0.001$). There was a significant synergistic effect between N rate and belowground interactions on the enhancement of wheat N uptake ($P < 0.01$) in the pot experiment. DI and DSI of mildew increased markedly with increasing N rate in both experiments (all $P < 0.001$). In the pot experiment DI and DSI showed no marked differences among belowground partitions (both $P > 0.05$) but belowground interactions had

Y. Chen · F. Zhang · P. Christie · L. Li (✉)
Key Laboratory of Plant-Soil Interactions, Chinese
Ministry of Education, China Agricultural University,
Beijing 100094, China
e-mail: lilong@cau.edu.cn

Y. Chen · F. Zhang · P. Christie · L. Li
Key Laboratory of Plant Nutrition and Nutrient
Cycling, Chinese Ministry of Agriculture, China
Agricultural University, Beijing 100094, China

Y. Chen · F. Zhang · P. Christie · L. Li
College of Resources and Environmental Sciences,
China Agricultural University, Beijing 100094,
China

L. Tang · Y. Zheng · Y. Li
College of Resources and Environmental Science,
Yunnan Agricultural University, Kunming 650201,
China

Y. Chen
Department of Soil Science and Agricultural
Chemistry, College of Resources and Environmental
Sciences, Sichuan Agricultural University, Ya'an
625014, China

P. Christie
Agricultural and Environmental Science Department,
Queen's University Belfast, Newforge Lane, Belfast
BT9 5PX, UK

different effects under different N rates, limiting disease occurrence under 0, 0.1 and 0.2 g N kg⁻¹ soil but promoting disease with 0.05 g added N kg⁻¹ soil. In the field experiment DI and DSI showed no significant differences between wheat monoculture and intercropping (both $P > 0.05$). However, the contributions of below- and above-ground interactions to disease control were different under different N rates, with interspecific root interactions increasing DI and DSI under different N rates and aboveground interactions increasing DI and DSI under zero-N application but decreasing DI and DSI at 150 and 300 kg N ha⁻¹. The data suggest that the microclimate in the field and biological control mechanisms due to belowground interactions in wheat–faba bean associations may influence the incidence and severity of wheat powdery mildew.

Keywords Wheat · Faba bean · Below-ground interactions · Nitrogen fertilizer · Powdery mildew

Introduction

Wheat yields in China increased from 0.8 t ha⁻¹ year⁻¹ on average in the 1950s to 3.7 t ha⁻¹ year⁻¹ in 2000 (Shen and Liang 2001). The main factor limiting increased wheat production is the severity of diseases such as stripe rust (*Puccinia striiformis*) and powdery mildew. In Yunnan Province, southwest China, wheat is the third most important cereal after rice and maize, with 0.6 million ha under cultivation (Yu et al. 2001) and yields averaging 2.1 t ha⁻¹, which is less than the national average, mainly due to the severity of wheat strip rust and powdery mildew (Shen and Liang 2001).

Powdery mildew is an important disease of wheat worldwide, especially in highly productive areas with a maritime or semi-continental climate (Bennett 1984). In China wheat powdery mildew used to occur only occasionally in the southwestern plateau and coastal area of Shandong Province before the 1970s (Tao et al. 1982; Liu 1989). However, its severity increased markedly during the early 1980s, mainly due to high application rates of nitrogenous fertilizers, production of semi dwarf wheat cultivars, and expansion of irrigated areas. The area of wheat

production affected by powdery mildew increased from 2.9 million ha in 1981 to 12.0 million ha in 1990 with an estimated grain yield loss of 14.4 million tons (Wu 1990). In recent years the productivity of wheat has been highly variable and this has been attributed mainly due to the severity of pests and diseases. Despite the adoption of several applications of the fungicide triadimefon at a cost of 215 yuan ha⁻¹ as the routine crop protection strategy (Zhu et al. 2004), effective integrated strategies for control of diseases such as powdery mildew include breeding wheat cultivars with resistance genes (Wang et al. 2005), growing mixtures of several wheat cultivars with different resistance (Browning and Frey 1969; Cox et al. 2004), intercropping wheat with other crop species such as maize, soybean or faba bean (Zhu et al. 2004), and improving cultural practices such as planting time, planting density, N fertilizer use and water control.

The epidemiology of wheat powdery mildew is influenced by a range of factors of which fertilizer use is the most important followed by pathogen source and climatic conditions. Large single applications or excessive multiple applications of N fertilizers have consistently resulted in serious outbreaks of fungal disease, and of powdery mildew in particular (Olesen et al. 2003). Some studies have demonstrated a significant influence of N fertilizer on powdery mildew, with a strong positive correlation between N rate and severity index of the disease and a significant negative correlation between grain yield and disease index (Zhang and Zhang 1991; Gao et al. 1993). Wheat grain yield and product quality were found to be substantially influenced by N nutrition (Guttieri et al. 2005; Varga et al. 2005) and foliar disease (Puppala et al. 1998), and N utilization and management are recognized as very important factors in wheat production (Olesen et al. 2000; Kratochvil et al. 2005). Numerous studies have indicated the advantageous effects of various cultural practices on disease control and higher yields, including mixtures of several varieties (Mundt 2002; Cox et al. 2004) or intercropping wheat with other species (Trenbath 1993; Zhu et al. 2004) and N fertilizer utilization associated with fungicide application (Varga et al. 2005). Cereal–legume intercropping is popular world-

wide and in China the main interspecific facilitation found in this cropping system was improved N nutrition of cereals from associated legumes through underground interactions (Li et al. 2001; Xiao et al. 2004). Numerous studies have focussed on competition and/or facilitation between intercropped wheat and faba bean for N (Xiao et al. 2004) or on the effects of N use on powdery mildew of wheat grown in monoculture (Olesen et al. 2000, 2003). However, to date there have been few studies on the relationship between N nutrition and powdery mildew as influenced by belowground interactions and N inputs in cereal–legume intercropping systems. The objectives of the present study were therefore to determine the effects of N application rate and belowground interactions on N nutrition of wheat, evaluate the influence of N rate and belowground interactions on wheat powdery mildew in wheat–faba bean intercropping systems under greenhouse and field conditions, and elucidate the relationship between plant N status and powdery mildew severity index.

Materials and methods

Pot experiment

The pot experiment was carried out from May to August 2004 in a greenhouse at Yunnan Agricultural University (YNAU), Kunming, Yunnan Province. The soil used was taken from the top 30 cm of the soil profile in a paddy field near YNAU, air-dried and ground, and sieved to pass through a 5-mm mesh. The nutrient status of the soil is shown in Table 1.

The experimental factors were four N rates (0, 0.05, 0.1, 0.2 g N kg⁻¹ soil designated N₀, N_{0.05}, N_{0.1}, N_{0.2}) and three belowground partitions (plastic film, 400-mesh nylon and no partition

between the wheat and faba bean root systems designated P, M, I) as shown in Fig. 1. There were four replicates, giving a total of 48 pots in a randomized block design. Basal P and K (0.15 g P₂O₅ and 0.15 g K₂O kg⁻¹ soil) were incorporated into the soil by mixing with 17 kg soil. The soil was then distributed among plastic plant pots 25 cm in height and 34 cm in diameter. One side of each pot was planted with 5 faba bean (cv. K324) seedlings and the other side with 15 wheat (cv. Yunmai 47) seedlings (Fig. 1) after seed surface-sterilization in 10% (v/v) H₂O₂ for 30 min and germination in saturated CaSO₄ solution for 12 h and in a porcelain tray in the dark for 48 h. The cultivars used were selected because they are commonly grown in the central part of Yunnan Province and were supplied by the Research Institute of Crop Resources of Yunnan Province. The wheat cultivar used (Yunmai 47) has moderate resistance to powdery mildew. The seedlings of both species were transplanted on 13 May 2004. Soil water content was regularly adjusted during the growth of the plants. No fungicides or insecticides were applied.

Field experiment

The field experiment was carried out at Lijia village, Zhongshu town, Luliang county, Yunnan Province during the 2004–2005 winter growing season. Bulk soil from the top 30 cm of the soil profile was collected, air-dried and sub-samples analyzed for selected physico-chemical properties and the results are shown in Table 1. The experimental design was a split-plot with three replicates in which the main plot treatments were 0, 150 and 300 kg N ha⁻¹ applied as urea and designated N₀, N₁₅₀ and N₃₀₀. The sub-plot treatments consisted of sole wheat (cv. Pan 93-1) and wheat/faba bean (cv. Fengdou No. 7) intercropping with or without belowground

Table 1 Nutrient status of the soils used in the two experiments

Experiment	pH (H ₂ O) (2.5:1)	Organic matter (g kg ⁻¹)	Total N (g kg ⁻¹)	Alkali-hydrolyzable N (mg kg ⁻¹)	Olsen P (mg kg ⁻¹)	NH ₄ OAc-extractable K (mg kg ⁻¹)
Pot	6.35	23.2	1.45	106.7	14.1	51.8
Field	7.24	14.2	1.22	57.1	15.5	30.3

Fig. 1 Schematic diagram showing the design of the pots in the greenhouse experiment with (a) no partition and (b) plastic film or nylon mesh partition between the roots of wheat and faba bean, and the design of the plots in the field experiment showing the layout of (c) monoculture

and (d) intercropping plots, and the positions of plastic film partitions or no partitions in the intercropping plots, and the positions of disease investigation points in both monoculture and intercropping plots. (+), wheat; (o), faba bean

partitions with plastic film between the roots of wheat and faba bean in the intercropping plots and designated W_m , W_p and W_i . The cultivars used here were local commonly grown cultivars and supplied by the Plant Protection Station of Luliang county and the wheat cultivar (Pan 93-1) has moderate resistance to powdery mildew.

The field had a long history of rice production and there was a firm ploughing pan at 30–40 cm depth. An impermeable plastic sheet was therefore inserted in the ground between the strips of wheat and faba bean to a depth of 0.5 m to prevent interspecific belowground interactions (Fig. 1d). The method of soil disturbance and plastic sheet insertion into the soil was as follows. In the intercropping plots (W_p and W_i) the soil where faba bean was to be grown was dug up and a ditch measuring 6 m long $\times$ 0.5 m deep $\times$ 0.6

(or 0.3) m wide) was dug. Plastic sheet was then either held against the wall of the ditch where belowground interactions were to be prevented or no plastic sheet was used where belowground interactions were to be allowed. The soil was then carefully replaced in each ditch, with the subsoil backfilled first and then the topsoil, which was finally compacted. This procedure was followed to ensure that the same degree of soil disturbance occurred in all the intercropping plots with and without belowground partitioning.

Seedbed dressings of 50% of the N rates and 100 kg P_2O_5 ha⁻¹ and 100 kg K_2O ha⁻¹ were evenly distributed by hand on the surface of the plots and then incorporated into the top 20 cm of the soil with hoes prior to sowing, and the remaining 50% of the N fertilizer was applied as a top dressing before the stem elongation stage on

a rainy day. No organic fertilizer was used. Wheat and faba bean were sown on 23 October 2004. The wheat seeds were drilled at a rate of 10 kg seeds ha⁻¹ with a row width of 0.2 m. Faba beans were dot-sown with a row spacing of 0.3 m and a plant spacing of 0.12 m. There were 28 rows of wheat in the sole wheat plots, and 1 row of faba bean:6 rows of wheat:2 rows of faba bean:6 rows of wheat:2 rows of faba bean:6 rows of wheat:1 row of faba bean in the intercropping plots. Plot size was 5.4 × 6 m (Fig. 1). Plots were irrigated with 30–60 mm (dependent on growth stage) on each time at emergence, tillering, stem elongation and flowering stages. No fungicide was used but the insecticides Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate 50 WP) at rate of 150 g a.i. ha⁻¹ in 750 l ha⁻¹ water and monocrotophos (*O,O*-dimethyl-*O*-(1-methyl-2-methylcarbamoyl)-vinyl phosphate 40 EC) at 750 ml ha⁻¹ in 1,125 l ha⁻¹ water were applied at stem elongation stage at flowering stage, respectively, as foliar sprays to control aphids.

Disease assessment

Powdery mildew on wheat occurring naturally and without inoculation of the pathogen was investigated at the flowering stage. In the pot experiment all green leaves on the main culms, a total of 45–60 leaves per pot, were investigated. In the field experiment 5 random points in half of the monoculture plots and 3 random points covering the border row and inner rows in half of the intercropping plots were selected (Fig. 1). At each point in sole wheat ten culms were randomly selected. In the intercropped wheat, ten culms at each row in border and inner rows were randomly selected. All green leaves on the selected culms, a total of 150–180 leaves and 270–320 leaves per plot, were investigated in the monoculture and intercropping plots, respectively. The severity of disease was estimated visually (Liu and Zhou 1988) based on the actual percentage of the total leaf area covered by powdery mildew. Each leaf was allocated a rating from 0 to 8, where 0 = no disease, 1 < 1.0% of leaf area diseased, 2 = 1.1–5.0%, 3 = 5.1–10.0%, 4 = 10.1–20.0%, 5 = 20.1–40.0%, 6 = 40.1–60.0%,

7 = 60.1–80.0%, and 8 = 80.1–100.0% (Zhu et al. 2004).

Soil and leaf sampling and analysis

Soil samples were taken prior to transplanting, air-dried and analyzed by standard soil chemical analysis methods (Bao 2000). Foliar samples were taken after disease investigation, oven-dried below 105°C for 30 min and then below 70°C for 48 h. Foliar N concentrations were determined on sub-samples ground to 1 mm by the micro-Kjeldahl procedure (Bao 2000).

Calculations and data analysis

Disease incidence (DI) and disease severity index (DSI) were calculated as follows:

$$DI(\%) = [(\sum N_i - N_0) / \sum N_i] \times 100 \quad (1)$$

$$DSI(\%) = [\sum (i \times N_i) / (8 \times \sum N_i)] \times 100 \quad (2)$$

where *i* represents disease categories 0, 1, 2, 3, 4, 5, 6, 7 and 8, and *N_i* is the number of leaves in each of the disease categories (Zhu et al. 2004).

Cahill (1999, 2002) discussed root competition for water and nutrients and shoot competition for light and interactions between below- and above-ground competition in some detail. He pointed out that in grassland ecosystems root and shoot competition do operate independently, but instead interact to affect plant growth. However, there is little information available on root and shoot interactions and their interactive effects in agricultural systems, and no studies have been reported on the separate contributions of below- and aboveground interactions to disease control. Accordingly, we made the assumption that the effects of below- and aboveground interactions are independent, and that the effect of intercropping is the sum of below- and aboveground interactions. The defence effects of intercropping and belowground interactions between wheat and faba bean on DI and DSI were calculated directly as follows:

$$DE_i = \frac{DI(DSI)_{w_i} - DI(DSI)_{w_m}}{DI(DSI)_{w_m}} \times 100\% \quad (3)$$

$$DE_b = \frac{DI(DSI)_{W_i} - DI(DSI)_{W_p}}{DI(DSI)_{W_p}} \times 100\% \quad (4)$$

Under field conditions it is very difficult or impossible to separate aboveground interactions between wheat and faba bean to eliminate wind-spread of pathogens among the plots, therefore the effect of aboveground interactions is not determined directly but is estimated indirectly as:

$$DE_a = DE_i - DE_b \quad (5)$$

where DE_i , DE_b and DE_a refer to the defence effect (%) of intercropping, belowground interactions and aboveground interactions in wheat–faba bean intercropping on DI or DSI, respectively; DI (DSI) is wheat powdery mildew incidence (severity index); W_m , W_p and W_i refer to sole wheat, intercropped wheat with plastic film partition between the roots of wheat and faba bean, and intercropped wheat with no root partition, respectively. $DI(DSI)_{W_i(W_m \text{ or } W_p)}$ refers to DI (DSI) of W_i (W_m or W_p).

Taking foliar N concentration as the independent variable (x) and DSI as the dependent variable (y), the best-fit nonlinear regression equation between foliar N concentration and DSI was established according to the distribution graph of values (x , y).

All data were subjected to analysis of variance using the SAS software package (Version 8.2, SAS Institute, Inc., Cary, NC, USA) and mean values were compared by calculating least significant difference (LSD) at the 5% level.

Results

Plant N status

In the pot experiment wheat foliar N concentrations were influenced significantly by both N application rate and belowground interactions (both $P < 0.001$). At all N rates foliar N concentrations in M (nylon mesh partition) and I (no partition) treatments increased substantially compared with the P (plastic film partition) treatment, but showed no difference between M and I

treatments. Average increases in wheat foliar N concentrations in M and I treatments compared with P treatment were 97.9, 39.6, 7.6 and 7.1% for N rates of 0, 0.05, 0.1 and 0.2 g kg⁻¹, and 20.2% averaged over all four N rates (Table 2), indicating that increasing wheat foliar N status due to belowground interactions between wheat and faba bean were largest in zero-N pots and decreased with increasing N application rate.

In the pot experiment wheat foliar N concentrations across all belowground partition types tended to increase with increasing N rate. Furthermore, the increase in foliar N attributable to N application was greater in the P treatments (by 181, 407 and 434% for $N_{0.05}$, $N_{0.1}$ and $N_{0.2}$, respectively) than in the M treatments (by 101, 173 and 190% for $N_{0.05}$, $N_{0.1}$ and $N_{0.2}$, respectively) or I treatments (by 96, 179 and 189% for $N_{0.05}$, $N_{0.1}$ and $N_{0.2}$, respectively) (Table 2). There was a significant interaction between N application and belowground interactions ($P < 0.01$).

Wheat foliar N concentrations in the field were influenced by N application rate ($P < 0.001$) but not by monoculture versus intercropping ($P > 0.05$). Because of heterogeneity in soil conditions in the field the differences between blocks were almost significant ($P = 0.057$).

In the field experiment foliar N concentrations showed no discernible differences between monoculture and intercropping irrespective of N rate and higher under intercropping by only 18.7, 7.5 and 0.7% at N_0 , N_{150} and N_{300} , respectively. Belowground interactions increased foliar N concentrations by only 11.0 and 15.2% at N_0 and N_{150} and decreased them by 5.7% at N_{300} . Overall, intercropping and belowground interactions increased foliar N concentrations of wheat by only 7.0 and 5.0% (Table 3). Foliar N concentrations in wheat increased markedly with increasing N rate across all planting patterns including intercropping with faba bean (Table 3).

Wheat powdery mildew

In the pot experiment DI and DSI were influenced significantly by N rate (both $P < 0.001$) but not by the degree of belowground interactions (both $P > 0.05$). DI and DSI increased substantially with increasing N rate across all degrees of

Table 2 Effects of N rate and belowground interactions on wheat foliar N concentrations (g kg⁻¹) in the pot experiment

Treatment	N ₀ ^a	N _{0.05}	N _{0.1}	N _{0.2}	Mean	LSD _{0.05}
Plastic film	3.6 (0.3)	10.0 (0.5)	18.0 (0.6)	19.0 (0.3)	12.6	1.3
Nylon mesh	7.0 (0.4)	14.1 (0.4)	19.2 (0.7)	20.3 (0.4)	15.2	1.5
No partition	7.0 (0.3)	13.8 (0.4)	19.6 (0.4)	20.3 (0.3)	15.2	1.1
Mean	5.9	12.6	18.9	19.9		0.7
LSD _{0.05}	1.1	1.3	1.8	1.1	0.6	

^a N₀, N_{0.05}, N_{0.1}, N_{0.2} represent 0, 0.05, 0.1, 0.2 g N kg soil⁻¹

Values are means of 4 replicates, standard errors are provided in parentheses

Table 3 Effects of N rate and planting pattern on wheat foliar N concentration (g kg⁻¹) in the field experiment

Treatment	N ₀ ^a	N ₁₅₀	N ₃₀₀	Mean	LSD _{0.05}
Wheat monoculture	13.0 (0.8)	22.0 (1.8)	25.4 (0.9)	20.1	4.9
Wheat–faba bean with plastic film partition	13.9 (0.7)	21.3 (1.2)	27.2 (0.7)	20.8	2.5
Wheat–faba bean with no partition	15.4 (0.8)	23.7 (0.9)	25.6 (1.2)	21.6	4.0
Mean	14.1	22.4	26.1		1.6
LSD _{0.05}	3.0	4.8	3.8	1.6	

^a N₀, N₁₅₀, N₃₀₀ represent 0, 150, 300 kg N ha⁻¹, respectively

Values are means of 3 replicates, standard errors are provided in parentheses

belowground interactions. Although there were no marked differences among belowground partition treatments across all N rates (Fig. 2), belowground interactions between wheat and faba bean had different effects on powdery mildew occurrence under different N rates, with belowground interactions (I compared with P) limiting powdery mildew occurrence under N₀, N_{0.2} and N_{0.1} (DI decreasing by 29.3, 15.6, 3.8% and DSI by 25.7, 16.0 and 0.9%), but promoting powdery mildew occurrence under N_{0.05} with DI and DSI increasing by 23.7 and 40.6% (Table 4).

In the field experiment DI and DSI were not influenced by monoculture versus intercropping (both $P > 0.05$) but were strongly affected by N rate (both $P < 0.001$) despite significant differences in DI and DSI between blocks ($P < 0.05$ and $P < 0.01$). DI values were <50% under N₀ but increased markedly to >90% with N₁₅₀ and N₃₀₀, DSI also increased substantially with increasing N rate across all planting patterns, while DI and DSI showed no significant differences between monoculture and intercropping under different N rates (Fig. 2).

Intercropping, belowground interspecific interactions and aboveground interactions made different contributions to the control of wheat

powdery mildew in the field experiment (Table 4). Belowground interactions had a positive effect across all N rates but there were no significant difference among N application rates. Aboveground interactions had a positive effect only at N₀ but showed negative effects at N₁₅₀ and N₃₀₀ and the differences between N₀ and the other two N rates were pronounced. Accordingly, at N₀ both below- and aboveground interactions had similar positive effects but application rates N₁₅₀ and N₃₀₀ showed marked differences between below- and aboveground interactions. Intercropping produced increases in DI and DSI values of intercropped wheat of 27.1 and 49.5% at N₀ but little change in DI at N₁₅₀ and N₃₀₀.

Relationship between foliar N concentration and powdery mildew severity index

The distribution graph of values (x , y) with different belowground partitions or planting patterns (monoculture vs. intercropping) showed significant positive relationships between N concentration and DSI, and the best-fit regression equations over different belowground partitions or planting patterns describing these synergistic relationships were $y = 0.0036x^{2.7768}$ ($R^2 = 0.7783$,

Fig. 2 Effects of N rate and belowground partitions or monoculture versus intercropping on powdery mildew incidence (DI) and powdery mildew severity index (DSI). Values are means of three (field experiment) or four (pot experiment) replicate treatments. Error bars are standard errors of means. Capital letters (A) indicate significant difference between planting patterns or belowground partitions ($P < 0.05$; Different lower case letters (a, b, c, d) indicate significant differences between N rates ($P < 0.05$). In the field experiment, N rates are 0, 150, 300 kg N ha⁻¹, respectively; planting patterns are W_m (wheat monoculture), W_p (intercropping wheat and faba bean with plastic film partition between the roots of two crops), W_i (intercropping wheat and faba bean with no partition between the roots of the two crop species), respectively. In the pot experiment, N rates are 0, 0.05, 0.1, 0.2 g N kg⁻¹ soil, respectively; belowground partitions between the two crop species are P (plastic film partition), M (nylon mesh partition), I (no partition), respectively

$P < 0.0001$) in the field experiment (Fig. 3a) and $y = 1.0333e^{0.1708x}$ ($R^2 = 0.8342$, $P < 0.0001$) in the pot experiment (Fig. 3b). The shapes of the best-fit curves were similar in the two experiments, with a clear break point indicating that when the N concentration was below the break point DSI was small and changed only slightly, but when the

N concentration was above the break point DSI increased markedly with increasing N concentration. The N concentration value of the break point was different in the two experiments, at 10.0 g kg⁻¹ in the pot experiment and 17.5 g kg⁻¹ in the field study (Fig. 3).

Discussion

Plant N status

As would be expected, in both the pot and field experiments wheat foliar N concentrations increased with increasing N application rate regardless of belowground interactions or whether wheat was grown in monoculture or intercropped with faba bean. The pattern of increasing foliar N concentration resulting from increasing N application, however, was different among N rates and belowground partitions in the pot experiment and between monoculture and intercropping in the field experiment, with larger increases in wheat foliar N status at lower N rates than at higher N rates in both experiments (Tables 2 and 3). The increases in foliar N were also larger with plastic film partitions than the mesh partitions or root intermingling in the pot experiment (Table 2), but very similar among the monoculture, intercropping and partition treatments in the field experiment (Table 3). Thus, there were substantial effects of belowground interactions on the enhancement of N nutrition of wheat in the pot experiment but not of intercropping or belowground interactions in the field. This may have been due mainly to the pots used being only 25 cm high and the confined space may have promoted the more pronounced belowground effects of intercropped wheat and faba bean observed in the pot experiment compared with field conditions.

Xiao et al. (2004) used a similar belowground partition method in a pot experiment and obtained similar enhancement of N concentrations in both wheat straw and grain from associated faba bean. However, to date there have been no reported studies using root barriers between wheat and faba bean under field conditions to compare with the present study. Li et al. (2003)

Table 4 Defence effect (%) of intercropping, below- and aboveground interactions of wheat–faba bean intercropping on wheat powdery mildew incidence and severity index

Upper values: defence effect or $LSD_{0.05}$ on DI;
lower values: defence effect or $LSD_{0.05}$ on DSI

Interspecific interaction	Pot experiment				Field experiment			
	N_0	$N_{0.05}$	$N_{0.1}$	$N_{0.2}$	N_0	N_{150}	N_{300}	$LSD_{0.05}$
Intercropping					27.1	–2.6	0.7	22.9
					49.5	7.3	0.3	42.4
Aboveground interactions					11.8	–8.0	–1.1	18.1
					24.0	–26.8	–19.6	49.2
Belowground interactions	–29.3	23.7	–3.8	–15.6	15.3	5.5	1.7	33.6
	–25.7	40.6	–0.9	–16.0	25.5	34.2	19.9	78.0
$LSD_{0.05}$					41.0	21.0	4.5	
					49.1	89.1	34.1

Fig. 3 Correlation of N concentration and powdery mildew severity index of wheat leaves in the field experiment (a) or in the pot experiment (b). W_m , W_p , W_i

in (a) and P, M, I in (b) are the same as Fig. 2. The break points are at the crossing points of the straight lines and curves

set up a micro-plot belowground partition experiment with maize–faba bean intercropping at Jinyuan county, Gansu Province, China, and found no significant differences in maize or faba bean plant N concentrations among three root partition treatments, but N uptake by faba bean or maize without any root partitions was about 20% higher than that with plastic sheet and nylon mesh barriers. Khanna (1997) and Bauhus et al. (2000) showed that acacia improved the N nutrition of eucalypts through N_2 fixation by the acacia and the decomposition of N-rich acacia roots and subsequent uptake by eucalypt fine roots of the N released. In the present pot experiment increased wheat foliar N concentrations were similar in treatments with mesh partitions and root intermingling, implying that soluble N compounds diffused or were transported from the rhizosphere of faba bean to the rhizosphere of wheat.

Influence of N addition on wheat powdery mildew

In agricultural ecosystems N application usually increases crop foliar fungal disease severity (Jenkyn 1976; Nordin et al. 1998; Olesen et al. 2003) but can sometimes potentially reduce disease severity (Huber and Watson 1974). Nitrogen addition has been hypothesized to increase foliar fungal disease severity by mechanisms such as increasing the concentration of foliar N available as a resource to the pathogens (the nitrogen-disease hypothesis) (Jensen and Munk 1997; Nordin et al. 1998; Strengbom et al. 2002), decreased production of defensive compounds (Sander and Heitefuss 1998), or increased microclimate humidity (Jenkyn 1976) (cited by Mitchell et al. 2003). In the present study, in both the pot and field experiments, wheat foliar N

concentrations increased significantly with increasing N rate and the DI and DSI of wheat were highly and positively correlated with the foliar N concentrations in accordance with the nitrogen-disease hypothesis (Jensen and Munk 1997; Nordin et al. 1998; Strengbom et al. 2002), implying that N fertilizer management may be a key component in the control of wheat powdery mildew.

Influence of belowground interactions on wheat powdery mildew

Based on the expectation that belowground interactions between wheat and associated faba bean may improve the N nutrition of wheat (Xiao et al. 2004) and that wheat powdery mildew is positively correlated with plant N status (Nordin et al. 1998; Strengbom et al. 2002), we hypothesized that belowground interactions between the two crop species would increase the DI and DSI of wheat in proportion to increasing foliar N concentrations. However, the results of the present study have not fully supported our hypothesis.

Overall, DI and DSI showed no significant differences among belowground partition treatments in either experiment (Fig. 2). In the pot experiment belowground interactions (I compared with P) led to wheat foliar N concentrations increasing by 98.0, 37.6, 8.7 and 7.0% for N_0 , $N_{0.05}$, $N_{0.1}$ and $N_{0.2}$, respectively (Table 2), associated with DI decreasing by 29.3, 15.6, 3.8% and DSI decreasing by 25.7, 16.0, 0.9% for N_0 , $N_{0.2}$, $N_{0.1}$, respectively, but DI and DSI increasing by 23.7% and 40.6% for $N_{0.05}$ (Table 4). In the field experiment belowground interactions increased wheat foliar N concentrations by 11.0 and 15.2% at N_0 and N_{150} and lowered them by 5.7% at N_{300} but the corresponding DSI values increased by 25.5, 34.2 and 19.9%, respectively (Table 4). This indicates that belowground interspecific interactions had different effects on wheat N nutrition and powdery mildew occurrence under different N rates, and there were considerable differences between the pot and field experiments in terms of which belowground interactions influenced wheat N nutrition and powdery mildew pathogen development. Dakora (2003) and Morgan et al. (2005) discussed biological control mechanisms such as

competition for nutrients, production of antibiotics and degradation of enzymes, antifungal compounds (defensive compounds) and phytoalexin accumulation, and induced resistance from bacterial or fungal symbiosis in the rhizospheres of mixtures of legumes and non-legumes. Although we do not fully understand the mechanisms involved in the present study, our observations imply that, in addition to plant N status, there were likely to be other factors that influenced the occurrence of powdery mildew in the presence of belowground interactions between wheat and associated faba bean and this merits further investigation.

Separate effects of below- and aboveground interactions and intercropping on wheat powdery mildew

As discussed above, belowground interactions between wheat and faba bean in the field promoted the occurrence of wheat powdery mildew under different N rates. The effects of aboveground interactions on powdery mildew in the field showed a clear tendency to promote the disease at N_0 but depress it at N_{150} and N_{300} (Table 4). This indicates that the effect of aboveground interactions on powdery mildew varies with different N application rates. One possible explanation is that changes in microclimate may explain the observations as follows. With no applied N, sole wheat and intercropped wheat were both deficient in N, plant growth was limited, and the microclimatic conditions in the sole wheat plots and the intercropped plots were similar, therefore the effects of aboveground interactions on DI and DSI may have been derived mainly from underground interactions which promoted wheat shoot growth and thereby indirectly influenced DI and DSI in contrast to changes in microclimate resulting from aboveground interactions. However, under application of 150 or 300 kg N ha⁻¹ the wheat plants grew in overcrowded conditions in both monoculture and intercropped plots but the microclimates differed between monoculture and intercropped plots due to increased velocity of air movements and correspondingly lower humidity, i.e. conditions less conducive to infection by and growth of the

powdery mildew pathogen in the intercropped plots compared with sole wheat. Similar explanations for experimental observations have been proposed by Carroll and Wilcox (2003) for grapevine powdery mildew and by Zhu et al. (2005) for rice blast. Because the effect of intercropping was considered as the sum of the effects of below- and aboveground interactions, DI and DSI of intercropped wheat compared with sole wheat increased only at N_0 but changed little at N_{150} and N_{300} and the differences due to intercropping between N_0 and N_{150} or N_{300} were significant (Table 4). These results have theoretical importance for understanding the separate contributions of below- and aboveground interactions in intercropping systems with different N application rates to influences on foliar diseases such as blast, rust and powdery mildew.

Relationship between foliar N status and DSI

Under both field and greenhouse conditions significant positive relationships were found between foliar N concentration and DSI (Fig. 3 a, b), although the forms of the best-fit regression equations were different. In the pot experiment a substantial increase in wheat foliar N concentration was associated with a very low DSI that changed little at N_0 (N: 3.1–8.2 g kg⁻¹; DSI: 0.9–4.5%) but with an exponential increase in DSI at $N_{0.05}$ (N: 8.8–14.7 g kg⁻¹; DSI: 4.6–26.4%) but a small increase in wheat foliar N concentration was associated with a substantial increase in DSI at $N_{0.1}$ (N: 17.1–20.4 g kg⁻¹; DSI: 12.4–38.4%) and $N_{0.2}$ (N: 18.5–21.2 g kg⁻¹; DSI: 26.3–54.8%). In the field experiment, however, a relatively large increase in wheat foliar N concentration was associated with a clear increase in DSI not only at N_{150} (N: 18.3–26.4 g kg⁻¹; DSI: 11.9–31.5%) and N_{300} (N: 21.9–30.7 g kg⁻¹; DSI: 16.1–44.0%) but also at N_0 (N: 11.7–18.9 g kg⁻¹; DSI: 1.7–10.5%). This indicates that wheat powdery mildew was very sensitive to N regardless of its source (e.g. N fertilizer application, atmospheric wet or dry deposition, or irrigation water).

There are several possible explanations for the differences in the results of the pot and field experiments. First, different cultivars of wheat and faba bean were used in the two experiments

and these may have had different nutritional and pathogen resistance characteristics. Second, the different edaphic and environmental conditions may have exerted different effects on plant growth in the greenhouse and in the field. Third, there may have been different effects of the belowground partitions in the two experiments. In the pot experiment the plastic film (P) would have prevented direct root contact, the P treatment would have prevented solute movement while in the nylon mesh (M) treatment limited root contact may have occurred and solute exchange would also have been possible between the two pot compartments. In the field experiment, however, plastic film to a depth of 0.5 m may not have completely prevented contact between some roots of wheat and faba bean and all solute movement. Fourthly, native soil N supply may have differed in the greenhouse and in the field. In addition, some N inputs may have occurred in the field from dry or wet atmospheric deposition (Diekmann and Falkengren-Grerup 2002) or from irrigation with river water. Because of this, even though the soil used in the pot experiment had higher fertility than that in the field experiment (Table 1), the degree to which N nutrition of wheat was improved by ambient N in the field experiment may have been larger than in the pot experiment, as evidenced by the similarity in the changes in foliar N concentrations and DSI of wheat leaves at N_0 in the field experiment and at $N_{0.05}$ in the pot experiment (Fig. 3).

Conclusions

Wheat foliar N was enhanced substantially by N addition in greenhouse and field conditions and also by belowground interactions between wheat and faba bean in the pot experiment. There was a significant synergistic effect between N rate and belowground interactions on the enhancement of wheat N nutrition. Wheat powdery mildew DI and DSI increased markedly with increasing N rate in both field and greenhouse experiments. In the pot experiment DI and DSI showed no marked differences among root partitions but belowground interactions between wheat and faba bean had different effects under different

N rates. This implies that, in addition to plant N status, there are likely to be other factors that influence the occurrence of powdery mildew in the presence of belowground interactions between wheat and faba bean and this merits further research. In the field experiment DI and DSI showed no significant differences among planting patterns. However, the contributions of above- and belowground interactions to disease control were different, with belowground interactions increasing DI and DSI under different N rates and aboveground interactions increasing DI and DSI under zero-N application but decreasing DI and DSI at 150 and 300 kg N ha⁻¹. This is likely to be associated with changes in microclimate due to aboveground interactions in N-fertilized intercropping plots.

Acknowledgements We thank the Science and Technology Department of Yunnan Provincial Government (Project 2003FCCFC01A009), Program for Changjiang Scholars and Innovative Research Team in University (project IRT0511), the National Natural Science Foundation of China (Project 30460061) and the Natural Science Foundation of Yunnan Province (Projects 2003C0040M and 2003C0041M) for generous financial support.

References

- Bao SD (2000) Soil and agricultural chemistry analysis. China Agriculture Press, Beijing, China, pp 30–268
- Bauhus J, Khanna PK, Menden N (2000) Aboveground and belowground interactions in mixed plantations of *Eucalyptus globulus* and *Acacia mearnsii*. Can J For Res 30:1886–1894
- Bennett FGA (1984) Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programs. Plant Pathol 33:279–300
- Browning JA, Frey KJ (1969) Multiline cultivars as a means of disease control. Annu Rev Phytopathol 7:355–382
- Cahill Jr JF (1999) Fertilization effects on interactions between above- and belowground competition in an old field. Ecology 80:466–480
- Cahill Jr JF (2002) Interactions between root and shoot competition vary among species. Oikos 99:101–112
- Carroll JE, Wilcox WF (2003) Effects of humidity on the development of grapevine powdery mildew. Phytopathology 93:1137–1144
- Cox CM, Garrett KA, Bowden RL, Fritz AK, Dendy SP, Heer WF (2004) Cultivar mixtures for the simultaneous management of multiple disease: Tan spot and leaf rust of wheat. Phytopathology 94:961–969
- Dakora FD (2003) Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytol 158:39–49
- Diekmann M, Falkengren-Grerup U (2002) Prediction of species response to atmospheric nitrogen deposition by means of ecological measures and life history traits. J Ecol 90:108–120
- Gao ZM, Tan GJ, Xu CY, Ma FZ, Li ZY (1993) Quantitative effects of sowing time, density and nitrogenous fertilizer on wheat powdery mildew. J Anhui Agric Univ 20:18–24
- Guttieri MJ, McLean R, Stark JC, Souza E (2005) Managing irrigation and nitrogen fertility of hard spring wheats for optimum bread and noodle quality. Crop Sci 45:2049–2059
- Huber DM, Watson RD (1974) Nitrogen form and plant disease. Annu Rev Phytopathol 12:139–165
- Jenkyn JF (1976) Nitrogen and leaf disease of spring barley. In: Fertilizer use and plant health. International Potash Institute, Worblaufen-Bern, Switzerland, pp 119–128
- Jensen B, Munk L (1997) Nitrogen-induced changes in colony density and spore production of *Erysiphe graminis* f. sp. *hordei* on seedlings of six spring barley cultivars. Plant Pathol 46:191–202
- Khanna PK (1997) Comparison of growth and nutrition of young monocultures and mixed stands of *Eucalyptus globulus* and *Acacia mearnsii*. Forest Ecol Manage 94:105–113
- Kratochvil RJ, Harrison MR, Pearce JT, Conover KJ, Sultenfuss M (2005) Nitrogen management for mid-Atlantic hard red winter wheat production. Agron J 97:257–264
- Li L, Sun JH, Zhang FS, Li XL, Yang SC, Rengel Z (2001) Wheat/maize or wheat/soybean strip intercropping. I. Yield advantage and interspecific interactions on nutrients. Field Crops Res 71:123–137
- Li L, Zhang FS, Li XL, Christie P, Sun JH, Yang SC, Tang CX (2003) Interspecific facilitation of nutrient uptake by intercropped maize and faba bean. Nutr Cycling Agroecosyst 65:61–71
- Liu XK (1989) Prospects of wheat powdery mildew research in China. Inf. Agric. Husb. August:1–10
- Liu XK, Zhou YL (1988) Study on investigating method of wheat powdery mildew. Chinese J Plant Protect 2:17–20
- Mitchell CE, Reich PB, Tilman D, Groth JV (2003) Effects of elevated CO₂, nitrogen deposition, and decreased species diversity on foliar fungal plant disease. Glob Change Biol 9:438–451
- Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant–microbe interactions in the rhizosphere. J Exp Bot 56:1729–1739
- Mundt CC (2002) Use of multiline cultivars and cultivar mixtures for disease management. Annu Rev Phytopathol 40:381–397
- Nordin A, Nasholm T, Ericson L (1998) Effects of simulated N deposition on understorey vegetation of a boreal coniferous forest. Funct Ecol 12:691–699
- Olesen JE, Mortensen JV, Jørgensen LN, Andersen MN (2000) Irrigation strategy, nitrogen application and fungicide control in winter wheat on a sandy soil. I. Yield, yield components and nitrogen uptake. J Agric Sci 134:1–11

- Olesen JE, Jørgensen LN, Petersen J, Mortensen JV (2003) Effects of rate and timing of nitrogen fertilizer on disease control by fungicides in winter wheat. I. Grain yield and foliar disease control. *J Agric Sci* 140:1–13
- Puppala V, Herrman TJ, Bockus WW, Loughin TM (1998). Quality response of twelve hard red winter wheat cultivars to foliar disease across four locations in central Kansas. *Cereal Chem* 75:94–99
- Sander JF, Heitefuss R (1998) Susceptibility to *Erysiphe graminis* f. sp. *tritici* and phenolic acid content of wheat as influenced by different levels of nitrogen fertilization. *J Phytopathol* 146:495–507
- Shen ZZ, Liang SS (2001) *Annals of China Agriculture*. China Agriculture Press, Beijing, China, pp 264–271
- Strengbom J, Nordin A, Nasholm T, Ericson L (2002) Parasitic fungus mediates change in nitrogen-exposed boreal forest vegetation. *J Ecol* 90:61–67
- Tao JF, Shen XZ, Qin JZ, Qin Y (1982) Studies on the resistance to powdery mildew in wheat species and varieties. *Acta Phytopathol Sinica* 12:7–14
- Trenbath BR (1993) Intercropping for the management of pests and diseases. *Field Crops Res* 34:381–405
- Varga B, Svečnjak Z, Maćešić D, Uher D (2005) Winter wheat cultivar responses to fungicide application are affected by nitrogen fertilization rate. *J Agron Crop Sci* 191:130–137
- Wang ZL, Li LH, He ZH, Duan XY, Zhou YL, Chen XM, Lillemo M, Singh RP, Wang H, Xia XC (2005) Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. *Plant Dis* 89:457–463
- Wu ZS (1990) Breeding for wheat disease resistance. In: Wu ZS (ed) *Wheat breeding*, China Agriculture Publishing Press, Beijing, pp 235–272
- Xiao YB, Li L, Zhang FS (2004) Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect ^{15}N techniques. *Plant Soil* 262:45–54
- Yu YX, Yang YH, Liu L (2000) Current situation and development strategy of wheat breeding in Yunnan Province. *Southwest China J Agric Sci* 13:91–97
- Zhang MP, Zhang HY (1991) Impact of fertilizer on wheat diseases. *Jiangsu Agric Sci* 2:39–41
- Zhu YY, Fang H, Wang YY, Fan JX, Yang SS, Mew TW, Mundt CC (2005) Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology* 95:433–438
- Zhu YY, Wang YY, Chen JB, Zhou HP, Li ZS, Li Y, Sun Y, Zhou KL, Wang M, Yang JC, Yang QH, Wang SM, Trevor H (2004) Crop diversity and disease control in wheat and faba bean. In: Zhu YY (ed) *Biodiversity for sustainable crop disease management: theory and technology*. Yunnan Science and Technology Press, Kunming, China, pp 461–470